

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of

Atty Ref.: 2752-52

MAERTENS, et al.

Group: Unassigned

Application No.: NOT YET ASSIGNED
(DIVISIONAL OF APPLICATION NO. 08/928,017)

Examiner: Unassigned

Filed: Herewith

For: PURIFIED HEPATITIS C VIRUS ENVELOPE
PROTEINS FOR DIAGNOSTIC AND THERAPEUTIC USE

July 6, 2001

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Preliminarily amend the above-identified application as follows.

IN THE SPECIFICATION:

Amend the specification as follows.

Insert the attached SEQUENCE LISTING before the claims pages and renumber subsequent pages accordingly.

Please insert the attached "ABSTRACT" after the claims pages.

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IN THE CLAIMS:

Amend the claims as follows.

Cancel claims 1-48, without prejudice.

Add the following new claims.

- 49. (new) A recombinant vector comprising a vector sequence, an appropriate prokaryotic, eukaryotic or viral promoter sequence followed by a nucleotide sequence allowing the expression of an HCV single or specifically oligomerized envelope viral protein selected from the group consisting of E1 and/or E2 and/or E1/E2 viral proteins.
50. (new) The recombinant vector according to claim 49, with said nucleotide sequence being characterised further in that it encodes a single HCV E1 protein starting in the region between amino acid positions 1 and 192 and ending in the region between amino acid positions 250 and 400, more particularly ending in the region between positions 250 and 341, even more preferably ending in the region between position 290 and 341.
51. (new) The recombinant vector according to claim 50, with said nucleotide sequence being characterised further in that it encodes a single HCV E1 protein starting in the region between amino acid positions 117 and 192 and ending in the region between amino acid positions 263 and 400, more particularly ending in the region between positions 250 and 326.
52. (new) The recombinant vector according to claim 50, with said nucleotide sequence being characterised further in that it encodes a single HCV E1 protein bearing a deletion of the first hydrophobic domain between positions 264 to 293, plus or minus 8 amino acids.

53. (new) The recombinant vector according to claim 49, with said nucleotide sequence being characterised further in that it encodes a single HCV E2 protein starting in the region between amino acid positions 290 and 406 and ending in the region between amino acid positions 600 and 820, more particularly starting in the region between positions 322 and 406, even more preferably starting in the region between position 347 and 406 and most preferably starting in the region between positions 364 and 406.
54. (new) The recombinant vector according to claim 53, with said nucleotide sequence being characterised further in that it ends at any of amino acid positions 623, 650, 661, 673, 710, 715, 720, 746 or 809.
55. (new) The recombinant vector according to claim 50, said nucleotide sequence further comprising operably linked a 5'-terminal ATG codon and a 3'-terminal stop codon.
56. (new) The recombinant vector according to claim 50, with said nucleotide sequence being characterised further in that a factor Xa cleavage site and/or 3 to 10, preferably 6, histidine codons have been added 3'-terminally to the coding region.
57. (new) A recombinant vector comprising any of the sequences as represented in SEQ ID NO 3, 5, 7, 9, 11, 13, 21, 23, 25, 27, 29, 31, 35, 37, 39, 41, 43, 45, 47 and 49, or parts thereof.
58. (new) The recombinant vector according to claim 49, further characterised in that at least one of the glycosylation sites present in said E1 or E2 protein has been removed at the nucleic acid level.

59. (new) A vaccine comprising a recombinant vector according to claim 59.
60. (new) A method of vaccinating a human comprising administering a a vaccine of claim 59.
61. (new) A composition comprising a recombinant vector according to claim 49.
62. (new) A host cell transformed with at least one recombinant vector according to claim 49, wherein said vector comprises a nucleotide sequence encoding HCV E1 and/or E2 and/or E1/E2 protein and a regulatory sequence operable in said host cell and capable of regulating expression of said HCV E1 and/or E2 and/or E1/E2 protein.
63. (new) A recombinant E1 and/or E2 and/or E1/E2 protein expressed by a host cell according to claim 62.
64. (new) A recombinant E1 and/or E2 and/or E1/E2 protein according to claim 63, further characterised in that said host cells are mammalian cells.
65. (new) A recombinant E1 and/or E2 and/or E1/E2 protein according to claim 63, further characterised in that said host cells are yeast cells.
66. (new) Method for purifying HCV envelope proteins selected from the group consisting of E1 and/or E2 and/or E1/E2, characterised as comprising at least the following steps:
- growing a host cell as defined in claim 62, transformed with said recombinant vector, in a suitable culture medium,
 - expressing said vector sequence under suitable conditions, and,
 - lysing said transformed host cells, preferably in the presence of an SH group blocking agent, such as N-ethylmaleimide (NEM),

- recovering said HCV envelope protein by affinity purification by means of for instance lectin-chromatography or immunoaffinity chromatography using anti-E1 and/or anti-E2 specific monoclonal antibodies, with said lectin being preferably lentil-lectin, followed by,
- incubation of the eluate of the previous step with a disulphide bond cleavage agent, such as DTT, preferably also in the presence of an SH group blocking agent, such as NEM or Biotin-NEM, and,
- isolating the HCV single or specifically oligomerised E1 and/or E2 and/or E1/E2 proteins by means of gelfiltration and possibly also by means of an additional Ni^{2+} -IMAC chromatography and desalting step.--

REMARKS

Claims 1-48 have been canceled, without prejudice.

Claims 49-66 have been added and are pending.

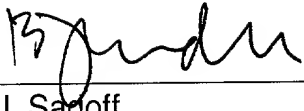
The attached paper copy of the SEQUENCE LISTING is the same as the paper and computer readable form of the SEQUENCE LISTING filed in the parent Application Nos. 08/612,973 and 08/928,017. No new matter has been added. Pursuant to Rule 822(e) no further computer readable copy of the SEQUENCE LISTING is believed required. A separate "Letter" is attached as required by the same Rule. The Office is requested to contact the undersigned if anything further is required at this time.

An early and favorable action on the merits is requested.

Respectfully submitted,

NIXON & VANDERHYE, P.C.

MAERTENS, et al. - Not Yet Assigned
(Divisional of Appln. No. 08/928,017)
Preliminary Amendment

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Variable	Mean	Standard deviation	Minimum	Maximum
Age	34.5	10.5	20	55
Gender	0.5	0.5	0	1
Marital status	0.5	0.5	0	1
Education	12.5	1.5	10	15
Income	15.5	5.5	10	25
Health status	0.5	0.5	0	1
Smoking status	0.5	0.5	0	1
Alcohol consumption	0.5	0.5	0	1
Exercise frequency	0.5	0.5	0	1
Stress level	0.5	0.5	0	1
Sleep quality	0.5	0.5	0	1
Work satisfaction	0.5	0.5	0	1
Life satisfaction	0.5	0.5	0	1
Overall health	0.5	0.5	0	1
Physical activity	0.5	0.5	0	1
Mental health	0.5	0.5	0	1
Social support	0.5	0.5	0	1
Work-life balance	0.5	0.5	0	1
Financial stability	0.5	0.5	0	1
Family harmony	0.5	0.5	0	1
Personal growth	0.5	0.5	0	1
Community involvement	0.5	0.5	0	1
Environmental awareness	0.5	0.5	0	1
Cultural appreciation	0.5	0.5	0	1
Artistic expression	0.5	0.5	0	1
Volunteer work	0.5	0.5	0	1
Charitable contributions	0.5	0.5	0	1
Philanthropic activities	0.5	0.5	0	1
Leadership roles	0.5	0.5	0	1
Networking opportunities	0.5	0.5	0	1
Professional development	0.5	0.5	0	1
Continuous learning	0.5	0.5	0	1
Adaptability to change	0.5	0.5	0	1
Resilience to stress	0.5	0.5	0	1
Emotional regulation	0.5	0.5	0	1
Conflict resolution skills	0.5	0.5	0	1
Effective communication	0.5	0.5	0	1
Team collaboration	0.5	0.5	0	1
Problem-solving abilities	0.5	0.5	0	1
Decision-making skills	0.5	0.5	0	1
Time management	0.5	0.5	0	1
Organization skills	0.5	0.5	0	1
Attention to detail	0.5	0.5	0	1
Proactive behavior	0.5	0.5	0	1
Initiative taking	0.5	0.5	0	1
Accountability	0.5	0.5	0	1
Responsibility	0.5	0.5	0	1
Integrity	0.5	0.5	0	1
Honesty	0.5	0.5	0	1
Trustworthiness	0.5	0.5	0	1
Reliability	0.5	0.5	0	1
Consistency	0.5	0.5	0	1
Stability	0.5	0.5	0	1
Endurance	0.5	0.5	0	1
Persistence	0.5	0.5	0	1
Determination	0.5	0.5	0	1
Commitment	0.5	0.5	0	1
Dedication	0.5	0.5	0	1
Passion	0.5	0.5	0	1
Enthusiasm	0.5	0.5	0	1
Optimism	0.5	0.5	0	1
Positivity	0.5	0.5	0	1
Hopefulness	0.5	0.5	0	1
Confidence	0.5	0.5	0	1
Self-belief	0.5	0.5	0	1
Self-efficacy	0.5	0.5	0	1
Self-esteem	0.5	0.5	0	1
Self-worth	0.5	0.5	0	1
Self-respect	0.5	0.5	0	1
Self-love	0.5	0.5	0	1
Self-care	0.5	0.5	0	1
Self-awareness	0.5	0.5	0	1
Self-reflection	0.5	0.5	0	1
Self-improvement	0.5	0.5	0	1
Personal growth	0.5	0.5	0	1
Self-actualization	0.5	0.5	0	1
Life purpose	0.5	0.5	0	1
Meaning in life	0.5	0.5	0	1
Sense of direction	0.5	0.5	0	1
Clarity of vision	0.5	0.5	0	1
Goal setting	0.5	0.5	0	1
Planning skills	0.5	0.5	0	1
Strategic thinking	0.5	0.5	0	1
Resourcefulness	0.5	0.5	0	1
Creativity	0.5	0.5	0	1</

The present invention relates to a method for purifying recombinant HCV single or specific oligomeric envelope proteins selected from the group consisting of E1 and/or E1/E2 characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulphide bond cleavage or reduction step is carried out with a disulphide bond cleavage agent. The present invention also relates to a composition isolated by such a method. The present invention also relates to the diagnostic and therapeutic application of these compositions. Furthermore, the invention relates to the use of HCV E1 protein and peptides for prognosing and monitoring the clinical effectiveness and/or clinical outcome of HCV treatment.